VARIATION IN *JUNIPERUS DURANGENSIS* AND RELATED JUNIPERS (CUPRESSACEAE): ANALYSIS OF nrDNA AND petN SNPs

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ABSTRACT

Recent discovery of a low shrub from Topia, in the state of Durango, Mexico that appears similar to both *J. durangensis* and *J. jaliscana*, prompted the analyses of nrDNA and petN-psbM (cpDNA) SNPs. The plants from Topia differed from *J. durangensis* by 2 indels but were shown to be closely related as shown in a minimum spanning network. *Phytologia 91(2): 353-358 (August, 2009).*

KEY WORDS: *Juniperus durangensis*, *J. monticola, J. martinezii, J. flaccida,* nrDNA, petN-psbM, SNPs, Cupressaceae, geographic variation.

Juniperus durangensis Mart. is a tree or large shrub to 5 m that generally branches near the base (Adams, 2008). It is often found on rhyolite, a nutrient poor rocky volcanic substrate, in the mountains of western Mexico from Sonora and Chihuahua southward to Aguascalientes. *Juniperus durangensis* is in the serrate leaf margined junipers and appears most closely related to *J. martinezii* Perez de la Rosa and then to *J. flaccida* Schlecht, *J. jaliscana* Mart. and *J. monticola* Mart. (Fig. 1).

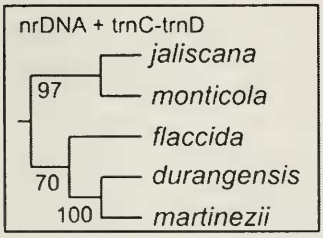

Figure 1. Clade from the serrate leaf margined junipers, based on nrDNA + trnC - trnD (cpDNA) data from Adams (2008) showing the putative relationship of *J. durangensis* to closely related junipers.

Recently, a low growing shrub was discovered near Topia, Durango that seems to be related to *J. durangensis*, although it has some characteristics of *J. jaliscana*. To further investigate the Topia juniper, sequencing of nrDNA and the petN-spacer-psbM cp DNA region were performed to obtain SNPs to reexamine the relationship of the Topia juniper to *J. durangensis* and other closely related junipers.

MATERIALS AND METHODS

Specimens collected (GenBank #: nrDNA; petN-psbM): *J. durangensis*, *Adams 6832-6834*, (FJ948469, FJ948473) 52 km w of El Salto, on Mex 40, Durango, MX; *Adams 11420-11421*, (FJ948469, FJ948473)Topia, Durango, MX;, *J. flaccida*, *Adams 6892-6893*, (FJ948470, FJ948476), on Mex. 60, 19 km E. of San Roberto Junction, Nuevo Leon, Mexico; *J. jaliscana*, *Adams 6846-6848*, (FJ948466, FJ948475), 19 km E of Mex. 200 on the road to Cuale, Jalisco, Mexico; *J. martinezii, Adams 5950, 5951, 8709,* (FJ948471, FJ948474)10 km s of Mex 85 on road to La Quebrada Ranch, Jalisco, MX; *J. monticola* f. *monticola*, *Adams 6874-6878*, (FJ948467, FJ746736) 1 km n of Mex 105, 9 km nw of Pachuca, El Chico National Park, Hidalgo, Mexico. Voucher specimens are deposited at BAYLU.

One gram (fresh weight) of the foliage was placed in 20 g of activated silica gel and transported to the lab, thence stored at -20° C until the DNA was extracted. DNA was extracted using the Qiagen DNeasy mini kit (Qiagen Inc., Valencia CA).

SNPs obtained from DNA sequencing

ITS (nrDNA) and trnC-trnD amplifications were performed in 50 µl reactions using 10 ng of genomic DNA, 3 units Qiagen Taq polymerase, 5 µl 10x buffer (final concentration: 50 mM KCl, 10 mM Tris-HCl (pH 9), 0.01% gelatin and 0.1% Triton X-100), 1.75 mM $MgCl_2$, 20 µl Q solution (2X final), 400 µM each dNTP, 1.8 µM each primer and 4%(by vol.) DMSO.

Gene	Primers	2x buffer	annealing	program	size bp
nrITS	ITS-42F/ ITSB+57R	K	50°C	(94-50x30)	1270-1272
petN	petN5F/psbM111R	E	50°C	(94-50x30)	839-845

Primers (5'-3'):

ITS: ITSA = GGA AGG AGA AGT CGT AAC AAG G;

ITSB = CTT TTC CTC CGC TTA TTG ATA TG.
ITSA and ITSB primers from Blattner (1999).
additional ITS primers (based on *Juniperus* sequences):
ITSA-42F = GAT TGA ATG ATC CGG TGA AGT
ITSB+57R = ATT TTC ATG CTG GGC TCT
petN - psbM:
petN5F = AAC GAA GCG AAA ATC AAT CA
psbM111R = AAA GAG AGG GAT TCG TAT GGA
petN and psbM primers were based on conserved sequences from *Juniperus* species.

The following PCR conditions were used: MJ Research Programmable Thermal Cycler, 30 cycles, 94°C (1 min.), 50°C or 57°C (2 min.), 72°C (2 min.), with a final step of 72°C (5 min.). The PCR reaction was subjected to purification by agarose gel electrophoresis (1.5% agarose, 70 v, 55 min.). In each case, the band was excised and purified using a Qiagen QIAquick gel extraction kit. The gel purified DNA band with the appropriate primer was sent to McLab Inc. for sequencing. Sequences for both strands were edited and a consensus sequence was produced using Chromas, version 2.31 (Technelysium Pty Ltd.). Alignments were made using MAFFT (http://align.bmr.kyushu-u.ac.jp/mafft/).

SNPs analyses

Aligned data sets (nrDNA and trnC-trnD) were analyzed by CLEANDNA (Fortran, R. P. Adams) to remove invariant data. Mutational differences were computed by comparing all SNPs, divided by the number of comparisons over all taxa (= Gower metric, Gower, 1971; Adams, 1975). Principal coordinate analysis was performed by factoring the associational matrix using the formulation of Gower (1966) and Veldman (1967). A minimum spanning network was constructed by selecting the nearest neighbor for each taxon from the pair-wise similarity matrix, then connecting those nearest neighbors as nodes in the network (Adams et al., 2003).

RESULTS AND DISCUSSION

Analyses of the nrDNA sequences revealed 26 mutational events that included a 2-bp indel (CA) that was present in the three *J.*

martinezii individuals and absent on all other taxa. In addition, one of the *J. flaccida* individuals (6893) contained an insertion (A) that was absent in all other samples. Thirteen of the mutational events were single events and 13 were multiple occurring with fidelity within populations. A minimum spanning network was constructed based on 13 SNPs (including one indel) and is shown in figure 2 (left). The Topia shrubs had no SNPs different from *J. durangensis*. Overall, these taxa appear to

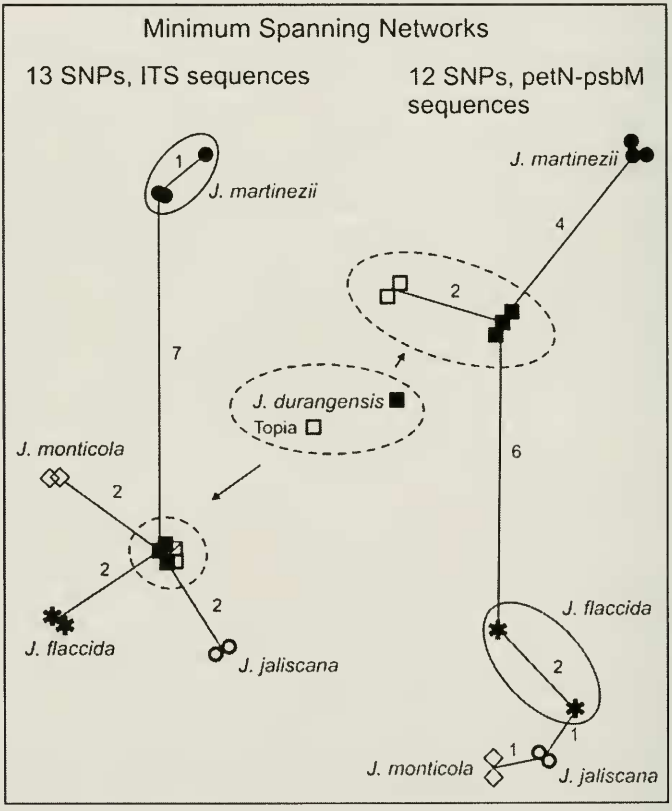

Figure 2. Minimum spanning network based on SNPs from nrDNA (left) and petN-spacer-psbM (right). The number of SNPs are next to the links.

be very closely related, with only *J. martinezii* having appreciable SNPs differences.

Analyses of a petN-spacer-psbM (from cpDNA) revealed 14 mutational events, with 6 of these being indels. Two events occurred in single individuals. Twelve SNPs (including 5 indels) were used to construct a minimum spanning network (Fig. 2, right). The Topia plants had 2 indels (an A at 401 and a deletion at 666) not found in *J. durangensis* (or other taxa). *Juniperus martinezii* is separated by 4 SNPs (Fig. 2, right) and *J. flaccida-J. jaliscana-J. monticola* are separated from *J. durangensis* by 6 or more SNPs.

Combining the nrDNA and petN-s-psbM data resulted in the minimum spanning network shown in figure 3. Notice that species are

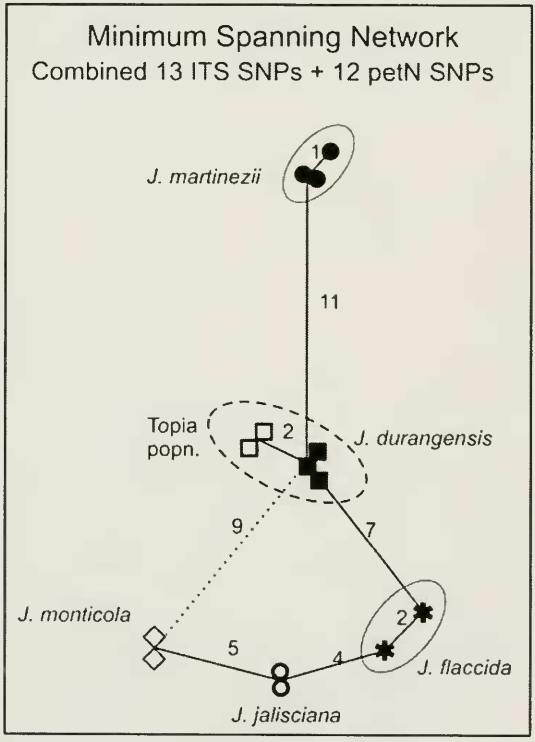

Figure 3. Minimum spanning network based on combined SNPs from nrDNA and petN-s-psbM sequencing. The dotted line is the second shortest link for *J. monticola* (9 SNPs).

separated by from 4 to 11 SNPs. The Topia plants are quite near typical *J. durangensis* in these two nucleotide sequences. However, it is clear that conclusions based on a single sequence might be misleading (cf. Fig. 2, left vs. right). Additional collections and analyses of the leaf essential oils of the Topia plants, as well as sequencing additional genes, should shed light on the scope of differentiation of this population and its affinities to other junipers.

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